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Induction of the mobile genetic element *Dm-412* transpositions in the *Drosophila* genome by heat shock treatment

(*copia*-like retroposon/temperature induction/pattern of localization/isogenic line)

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ABSTRACT **Males of a *Drosophila melanogaster* isogenic line with a mutation of the major gene for *radius incompletus* (ri) were treated by standard light heat shock (37°C for 90 min) and by heavy heat shock (transfer of males from 37°C for 2 hr to 4°C for 1 hr and back; this procedure was repeated three times). In the F_1 generation of treated males mated with nontreated females of the same isogenic line, mass transpositions of *copia*-like mobile genetic element *Dm-412* were found. The altered positions of the element seem nonrandom; five "hot spots" of transposition were found. Probabilities of transpositions were estimated after light heat shock and heavy heat shock and in the control sample. These probabilities were, respectively, 3.4×10^{-2}, 8.7×10^{-2}, and $<4.1 \times 10^{-4}$ transpositions per genome per occupied position per generation. Therefore, as a result of heat shock treatment, the probabilities of transpositions were two orders of magnitude greater than those of the control sample in the next generation after induction. Comparison of the results with those after stepwise temperature treatment shows that the induction depends on the intensity of the stress action (temperature treatment) rather than on the type of the stress action.**

In recent years information about the temperature sensitivity of mobile genetic elements (MGEs) has accumulated. Vasilyeva and coworkers (1–5) discovered the effects of stepwise temperature treatment of *Drosophila* at specially sensitive periods of the pupal stage. The heritable changes of expression of the mutant *radius incompletus* (ri) were seen accompanied by the changed patterns of localization of *copia*-like MGEs. Junakovic *et al.* (6) used Southern blotting with probes containing fragments of *copia*-like MGEs to show that heat shock (HS) of flies causes restriction fragment length polymorphism among fragments containing the MGE copies.

These data agree with the results of sequencing the different MGEs and of investigating the dynamics of their transcription and transposition. Many MGEs, including *copia*-like ones, contain sites similar to enhancers (7) and HS regulation sites (8, 9). After HS treatment of flies, transcription and transposition of different MGEs seemed to be activated (9–11).

From this evidence, speculations and hypotheses have been advanced about the genetic system of HS regulation in relation to heritable temperature effects in *Drosophila* (4, 5, 10–12). There have also been theories about the evolutionary importance of the genome system of MGE genomic patterns after environmental stress (temperature, etc.) (4, 5, 10–12).

However, the data are incomplete. Vasilyeva and coworkers (1–3, 13) in most of their work used heterogeneous *Drosophila* lines, and *in situ* hybridization was done many tens of generations after temperature treatment. Indeed, in some repeated experiments this interval was decreased to several generations with the same results. But the possibility of selective influence was not excluded completely. Junakovic *et al.* (6) found the effects in the next generation, but their restriction fragments were not compared with patterns of MGE localization in polytene chromosomes.

In this paper (see also ref. 14), we present data that demonstrate multiple transpositions of *copia*-like MGE *Dm-412* in the next generation after HS treatment. This work was done with an isogenic *Drosophila* line to decrease pattern variability in the control sample and after treatment.

MATERIALS AND METHODS

Isogenic Lines. The isogenic lines with a mutation of the major gene for ri were created by using marked balanced chromosomes (15): *M5* (X chromosome), *Cy/Pm* (chromosome 2), *D/Sb* (chromosome 3). Of 20 isogenic lines, one (N 49) was used for the HS treatment, being most stable in its pattern of MGE *Dm-412* localization and expression of the character ri.

Control Sample. Young males and virgin females were collected from mass cultures of isogenic line N 49 and were placed at 25°C in separate cultures in the proportion of one male/two females (Fig. 1). Descendants of these crosses were used as a control sample. A total of 134 larvae was used: 106 represented general control, and 28 were individual controls for males treated by light HS (LHS). These latter larvae were from the crosses of these males before they were treated by HS. Table 1, column 2 contains data for all control larvae where the patterns were completely stable.

LHS Treatment (Fig. 1). Hundreds of males of 2–4 days of age, hatching out from mass culture, were treated for 90 min at 37°C. All males survived. On the fourth to fifth day after treatment, males were individually crossed with two to five virgin nontreated females of the same line, N 49. The males were transferred to new untreated females at intervals of 4–5 days several times. As a result we had many repeated crosses for the same treated males. The larvae of the next generation (F_1) were used for the preparation of salivary gland polytene chromosomes. It is important to underline that these paired chromosomes are compounds of chromosomes, one treated by HS and the other nontreated.

Heavy HS (HHS) Treatment. HHS treatment was used to intensify the effect. Two hundred males of 2–4 days of age after hatching were treated at 37°C for 2 hr, followed by 1 hr at 4°C, repeated three times (see Fig. 1). There was mass death of the males. In the first experiment, only 10 males survived, but 7 were sterile and 3 were fertile (see A49, G49, and B49 in Table 1). Additional males were obtained in repeated HHS experiments. The descendants of these males

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Abbreviations: MGE, mobile genetic element; HS, heat shock; LHS, light heat shock; HHS, heavy heat shock; ri, radius incompletus mutant.

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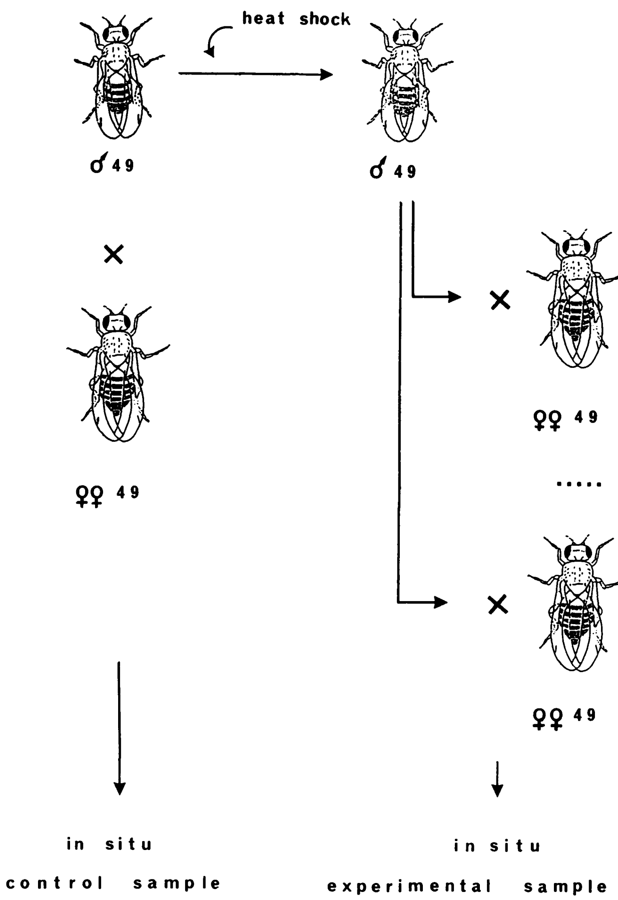

FIG. 1. Scheme of crosses and HS treatment.

with nontreated females were produced by the same procedure as for LHS.

Hybridization of Cloned DNA *in Situ*. This hybridization was by the standard method (16) used earlier (2, 3). DNA of MGE *Dm-412* (*mdg-2*) was from an original line of Finnegan *et al.* (17) cloned in pBR 322 with a *Bam*HI restriction site. Standard exposure time was 7–10 days. In addition to the intensively labeled bands in the chromosomes we also found some heterogeneous sites of weak hybridization in the control and experimental samples at chromosome positions 62DE, 62F, 85DI, 86D, and 86F. After prolonged exposure, these hybridization sites were expressed more strongly. Probably these positions correspond to segments of intercalary heterochromatin that is underreplicated during the formation of polytene chromosomes, and so these segments hybridized poorly with the probe (18, 19). Another possibility is that these hybridization sites correspond to MGEs of lesser homology with the probe. We excluded these positions from the patterns scored in both control and treated individuals because of the instability and weakness of their expression.

Examples of labeled chromosome preparations from control crosses and after HS are shown in Fig. 2. Both preparations are from the descendants of one male. The alternative positions of MGE localization after HS are indicated.

RESULTS AND DISCUSSION

The main results of the experiment are given in Table 1, where column 2 corresponds to control crosses (134 larvae), columns 3–6 correspond to compounds of the F_1 after LHS, and columns 7–15 correspond to compounds of F_1 after HHS. All patterns of control larvae labeled chromosomes are identical. The patterns of descendants of some treated males after LHS and HHS are similar, but the proportions of such males are very different: four unchanged patterns among five flies for LHS, and one unchanged pattern among nine flies for HHS. This could be the consequence either of more intensive action of HHS on frequency of transpositions or of selection during HHS treatment of males with rearranged MGE patterns. The second hypothesis seems very unlikely.

All descendants of treated males and nontreated females of the same isogenic line N 49 evidently have compound polytene chromosomes containing one homologue from the treated male and another one from the nontreated female. Therefore, in this experiment we can identify only transpositions—i.e., altered positions of MGE sites but not excisions. Actually, we had no losses of the site in Table 1. But males of the F_1 have haploid polytene X chromosomes untreated by HS. Therefore, these males do not contain transpositions and excisions in the X chromosome. Actually in our F_1 samples there were both males and females, but the latter predominated. These female flies showed some transpositions in the X chromosome (2B, 12B), but the number of X chromosome transpositions is much less than that in other chromosomes.

It is evident that on the background of unchanged MGE pattern of genomes from control crosses induced transpositions were discovered. Five of these induced transpositions were seen in descendants of most treated males after both LHS and HHS (positions 43B, 49CD, 56A, 56E, and 66A). This result means that sites of the induced transpositions are not random. Probably these positions are equivalent to the "hot spots" of transposition of Gvozdev-Kaidanov (20). Other sites of transposition are dispersed randomly. We intend to analyze carefully the data in Table 1 in a forthcoming paper.

We can estimate quantitatively the magnitude of transposition induction after LHS and HHS. We assume that all altered positions of MGEs in the daughter generation appear by independent and random transpositions. The number of transpositions should be proportional to the number of occupied positions and to the portion of free accessible positions. Then, as shown by Kapitonov *et al.* (21), the average number of transpositions per genome per generation per occupied position is

$$\Delta n = \lambda n \frac{(m - n)}{m},$$

where m is the total number of possible MGE localizations in the genome, n is the number of occupied positions before induction, and λ is the probability of transposition. From the summary data for all our ri-lines and populations [control line, "selection," and "temperature" lines (2, 3), isogenic lines, etc.], the total positions where the *Dm-412* has ever been found is not <86. We accept this figure as the lower estimate of the possible maximum positions ($m = 86$). The observed number of occupied positions in the control sample (isogenic line N 49) (n) equals 26 (see Table 1); data for LHS and HHS are shown in Table 2. Then for LHS $\lambda = 3.4 \times 10^{-2}$ transpositions per genome per occupied position per generation, and for HHS $\lambda = 8.7 \times 10^{-2}$ transpositions per genome per occupied position per generation. These figures differ, showing that the probability of transpositions depends on HS intensity.

From the control sample, we can estimate the upper bound of the probability of noninduced transpositions. The patterns of 134 control larvae have no heterogeneous positions. Hence, the number of transpositions per genome is $\Delta n < 1/134$. Then with the same estimates of m and n as before we have $\lambda < 4.1 \times 10^{-4}$ transpositions per genome per occupied position per generation of isogenic line N 49. In other words, the probability of transposition was increased by more than two orders of magnitude as a result of HS.

If the transpositions depend on one another (clusters of transpositions), the estimate of λ would be lower, but there

Table 1. Results of *in situ* hybridization of probe containing MGE *Dm-412* (*mdg-2*) with larval polytene chromosomes of isogenic line N 49 of *Drosophila melanogaster* in the control sample and after HS (F_1)

		LHS					HHS								
Chromosome segment	Control (134)	J49 (13)	E49 (13)	I49 (12)	S49 (6)	P49 (5)	A49 (12)	G49 (11)	B49 (42)	M49 (20)	P49 (25)	O49 (19)	C49 (16)	F49 (24)	D49 (23)
2B										11*					
6F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12B											10*				
16F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18EF	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
32CD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
39C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
42E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
42F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
43B		7*						4*	11*	9*	8*	9*	9*	11*	3*
45D	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
49CD		7*						6*	15*	9*	8*	9*	9*	11*	3*
56A		2*						2*	3*	3*	2*	4*	4*	4*	
56E		2*						2*	3*	3*	2*	4*	4*	4*	
60C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
63A												3*			
64C												3*			
65A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
65E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
66A		5*						2*	13*	9*	6*	5*	2*	4*	2*
67DE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
70E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
75AB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
76A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
79E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
82E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
85D		7*													
88E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
88F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
90B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
92C										4*	6*			7*	
96A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
98E									16*						
98F									16*						
99B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Letters (such as A49) designate individual males of line N 49. Above each column are shown (in parentheses) the numbers of descendant larvae of each male in F_1. Number of control larvae is 134. All positions designed + are occupied by the control sample and by all F_1 larvae. Asterisks (*) with numbers indicate the appearances of alternative positions of MGE (transpositions). Column 2 corresponds to the control sample, columns 3–7 correspond to LHS, and columns 8–16 correspond to HHS. We include in the control sample 106 larvae of general control for isogenic line N 49 and 28 larvae corresponding to the designated males before LHS.

is no doubt that HS sharply increases the probability of transpositions in the next generation—i.e., HS is a direct causal factor of transposition.

In our previous work (1–5, 13, 22) we used a different method of temperature treatment, stepwise change of culture temperature (29°C → 18°C). This treatment of the control population also induces transposition, although this result was found after many tens of generations. These transpositions also were located nonrandomly, but the spectra of transposition overlapped only partially with ones induced by HS. The MGE pattern of the control isogenic line N49 contains 26 sites, 22 of which coincided with the pattern of initial control population ri^c (see refs. 2 and 3). In the experiments with HS of line N 49, there were 13 different positions, and only 6 positions among them coincided with transposition sites induced by the stepwise temperature treatment of the ri^c population temperature lines ri^{c113} and ri^{c149} (2, 3). Perhaps these differences reflect the properties of different treatments. After stepwise treatment the transposition spectra depended on the time period of development but not after HS. Therefore, we do not expect these spectra of induced transpositions to be the same.

Clearly stepwise treatment is less stressful than LHS and much less stressful than HHS. The effect of stepwise treatment is revealed only under very specific conditions of narrow sensitive periods of the pupal stage when the phenotypic effect at F_0 is maximum and, therefore, visible. Much less specific conditions are required for LHS and HHS, and the result is achieved by increasing the intensity of treatment. For temperature induction of transpositions the stress intensity seems to be important, being able to initiate the HS regulatory system and being compatible with viability. The particular temperature is not so important. Low-stress intensity can be partially compensated for by the increased sensitivity of the genome at specific periods of development.

Interestingly we had no success in inducing transpositions by stepwise treatment (29°C → 18°C) at the sensitive periods of the pupal stage of isogenic lines. In the four isogenic lines

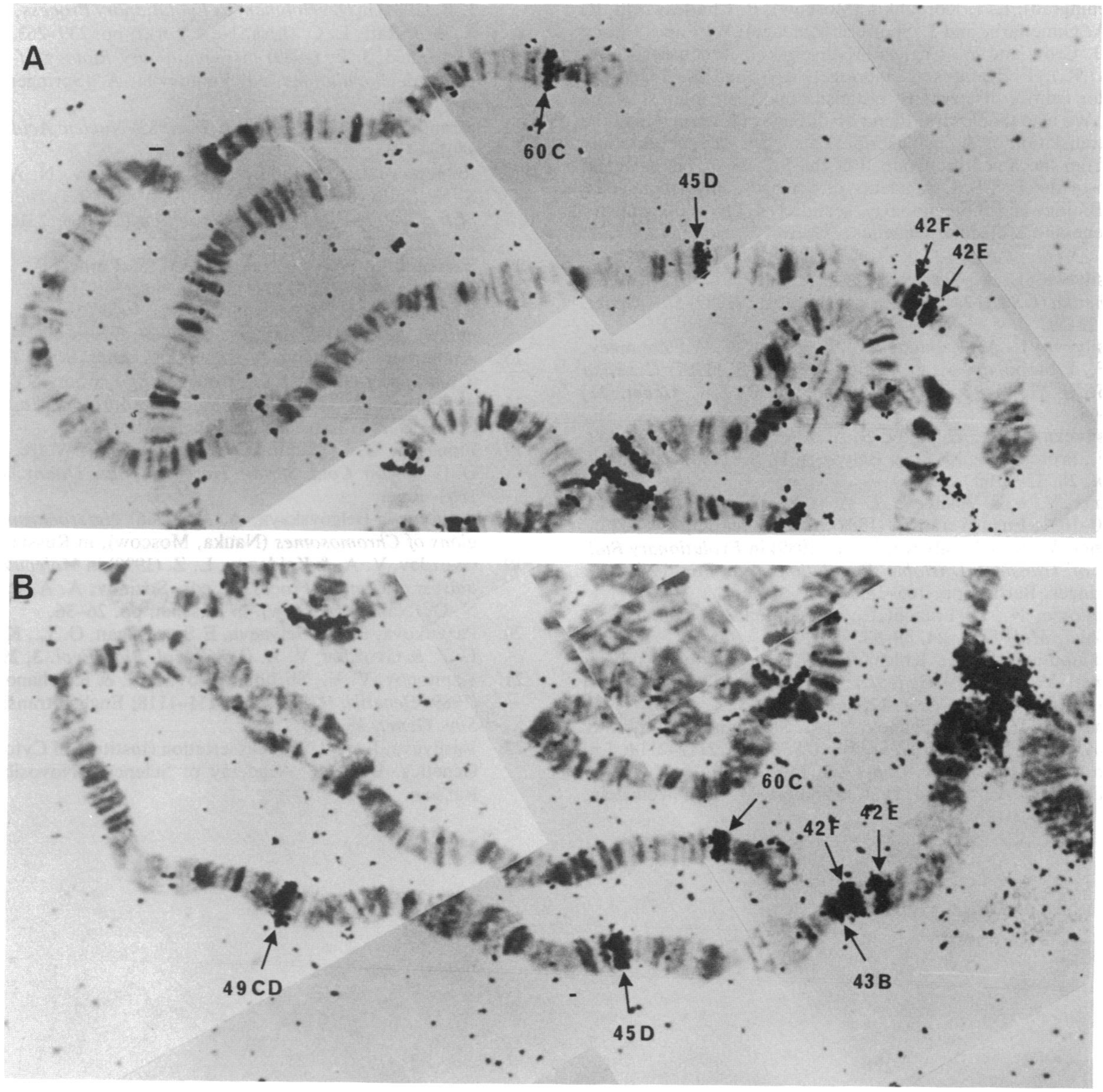

FIG. 2. Results of *in situ* hybridization of the probe containing MGE *DM-412* (*mdg-2*) with *Drosophila* polytene chromosomes of isogenic line N 49. (*A*) Descendant of nontreated male and female. (*B*) Descendant of HS-treated males and nontreated females at F_1. Data are from the same male (J49, LHS). Sites 43B and 49CD are absent before treatment (*A*) and present after LHS (*B*).

(NN 2, 27, 43, and 49) the patterns of MGE *DM-412* localization were the same in the next generation. The phenotypic changes after treatment disappeared in later generations.

We believe these negative results to be quite understandable. The high nonrandomness of localization of the temperature-induced transpositions, shown here and earlier (2–5, 13, 22), mean that there are very restricted and different possibilities of induced transpositions in different isogenic lines, having two to three times more "narrow" patterns of DM-412 localization (in comparison with the control population ri^c) (2, 3). Many positions in DM-412 are unchanged. The predominant group of isogenic lines might be predicted to yield stable patterns that are of low sensitivity to temperature treatment.

Table 2. Estimates of probability of transpositions in control sample and after LHS and HHS

Sample	Larvae, no.	Alternative positions, no.	Δn per genome per generation	λ transpositions per genome per generation per occupied position
Control	134	<1	<0.0075	$<4.1 \times 10^{-4}$
LHS	48	30	0.625	3.4×10^{-2}
HHS	192	303	1.58	8.7×10^{-2}

Furthermore, the necessary conditions for stabilization of alternative temperature lines suggest that similarly located transpositions are induced only in the very synchronized cultures. In the opposite case, individuals with changed patterns would be rare, and these patterns would be mixed after some generations of crossing and so would be lost; this loss results in the restoration of initial state and phenotype. Earlier (5, 22) we had underlined that such dynamics were predominant after stepwise temperature treatment.

Therefore, some negative results are completely expected and predictable and are not an argument against the reality of temperature induction. The basic finding is the example of an isogenic line that expresses this phenomenon significantly and clearly.

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